UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/462,635	04/10/00	SCHMIDT	G 020600-285

021839 HM12/0518
BURNS DOANE SWECKER & MATHIS L L P
POST OFFICE BOX 1404
ALEXANDRIA VA 22313-1404

EXAMINER	
GOLDBERG, J	
ART UNIT	**PAPER NUMBER**
1655	12

DATE MAILED: 05/18/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/462,635	Applicant(s) SCHMIDT ET AL.	
	Examiner Jeanine A Enewold Goldberg	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *three* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 March 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *4,5,7-12 and 14-63* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *4,5,7-12 and 14-63* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) ☒ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other: .

DETAILED ACTION

1. This action is in response to the papers filed March 7, 2001. Currently, claims 4-5, 7-12, 14-63 are pending. All arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow.

2. Any objections and rejections not reiterated below are hereby withdrawn.

3. All previous grounds of rejection have been withdrawn in favor of the new grounds of rejection. Thus, the arguments are moot in view of the new grounds of rejection.

New Grounds of Rejection

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 4-5, 7-12, 25 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A1) Claims 4-5, 7-12, 25 are indefinite over the recitation "a terminal" because it is unclear what a terminal of each nucleic acid is. While the termini of the DNA molecule is a recognized term, it is unclear what "terminals" include.

B1)

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

5. Claims 4-5, 7-12, 25-36 are rejected under 35 U.S.C. 102(e) as being anticipated by Rothberg et al. (US Pat. 5,871,697, February 16, 1999).

Rothberg et al (herein referred to as Rothberg) teaches a method for categorizing nucleic acid by

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein the endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has sticky ends of a known base sequence and of a known common length (col. 9, lines 42-43);

(ii) contacting the nucleic acid population with an adaptor to ligate the adaptor to a termini of each nucleic acid in the population such that the adaptor has a double stranded primer portion having a known base sequence and a single stranded portion complementary to the known sticky end of the nucleic acids of the population (col. 9, lines 43-56);

(iii) contacting the nucleic acid with one or more oligonucleotide sets (57-60) and

(iv) categorizing the nucleic acid by isolating nucleic acid which correctly hybridizes to an oligonucleotide set, wherein each oligonucleotide sequence in each oligonucleotide set has a pre-determined recognition sequence such that the recognition sequence is situated in the portion of the nucleic acid which was double stranded after digestion with the endonuclease (limitations of Claim 4, 7).

Simply, Rothberg teaches that following cDNA preparation, the next step is simultaneous RE cutting of and adapter ligation to the sample cDNA sequences (col. 48, lines 42-44). As seen in Figure 2D the oligonucleotide 222 comprises a segment complementary to the adaptor, the overhang/sticky end, the restriction endonuclease site and the double stranded nucleic acid (limitations of Claim 5, 8). Rothberg teaches that primers are preferably constructed with a subsequence 226 of P nucleotides. Length P is preferably from 1 to 6 and more preferably either 1 or 2 (col. 51, lines 49-56)(limitations of Claim 9, 10, 11). Rothberg teaches that if necessary, prior to the first step, the cDNA sample is prepared by methods commonly known in the art, such as amplification (col. 47, lines 23-26 and col. 87, lines 15-31)(limitations of Claim 12). Rothberg teaches that the primer comprises at the 3' end of and contiguous with the longer strand sequence the portion of the restriction endonuclease recognition site remaining on the nuclei acid fragment terminus after digestion by the restriction endonuclease...contiguous to said one or more additional nucleotides, and optionally such that said primers comprising a particular said one or more additional nucleotides can be distinguishably detected from said primers comprising a different said one or more additional nucleotides (col. 11, lines 20-39). Rothberg teaches why a primer

complementary to a portion of the double-stranded nucleic acid is preferable "the joint result of using primers 223 with subsequence 226 in multiple PCR reactions after one RE/ligase reaction is to extend the effective target subsequence from the RE recognition subsequence by concatenating onto the recognition sequence a subsequence which is complementary to subsequence 226 (limitations of Claim 37). Thereby, many additional target subsequences can be recognized while retaining the specificity and exactness characteristic of the RE embodiment (col. 52, lines 6-14). Rothberg explicitly teaches that restriction enzymes (RE's) such as those known as class IIS restriction enzymes, which produce overhangs of unknown sequence are less preferable (col. 41, lines 12-15). Rothberg teaches that preferred REs have a 6 pb recognition site and generate a 4 bp 5' overhang. The RE embodiments are also adaptable to a 2 bp 5' overhang, which is less preferred since 2 bp overhangs have a lower ligase substrate activity than 4 bp overhangs (col. 42, lines 5-9). Rothberg specifically teaches that adapter 250 is specific for the RE BamHI, as it has a 3' end complementary to the 5' overhand generated by BamHI (col. 45, lines 64-65). Similarly, Adapter 251 is specific for HindIII.

Rothberg also teaches a kit which contains one or more restriction endonucleasese, adapters and primers of the instant invention (col. 25-26)(limitations of Claim 26-29, 32-36). Rothberg teaches that the primers are detestably labeled such that primer with differing said one or two additional nucleotides have different labels that can be distinguishably detected (col. 26, lines 30-32).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 7-8, 26-33, 35-38, 41 rejected under 35 U.S.C. 103(a) as being unpatentable over Sibson (WO 94/01582, January 1994) in view of Rothberg (US Pat. 5,871,697, February 16, 1999).

It is noted that Claims 7 and 50 do not require that a known base sequence is provided, merely that a known common length is known.

Sibson teaches a method of categorizing nucleic acid. Sibson teaches a method which comprises producing a nucleic acid population by action of a endonculease on double-stranded nucleic acid, such that each nucleic acid in the nucleic acid population has a double stranded portion (pg. 13, step a). Suitable restriction endonucleases which recognize single stranded DNA and which also leave a cleaved sequence overhang when cutting double stranded DNA include BstNi, DdeI, HgaI, HinfI and MnlI (pg. 11, lines 26-27)(limitations of Claim 3). Preferred reagents or Class II restriction endonucleases that cleave sites which are asymmetrically spaced across two strands of DNA and the specificity of which is not affected by the nature of the bases adjacent to a cleavage site (pg. 16, lines 1-3)(limitations of Claim 2, 6 25, and 36). Examples of these endonucleases may leave a 5 base overhang starting 5 bases from the cut site or cut 7

bases away leaving only a 1 base overhang (pg. 12, lines 2-4)(limitations of Claim 35). The nucleic acid population is contacted with an adaptor to ligate the adaptor to a terminal of each nucleic acid in the nucleic acid population such that the adaptor comprises a double stranded primer portion having a known base sequence and a single stranded portion complementary to the known sticky end of the nucleic acids in the nucleic acid population (pg. 13, step b)(limitations of Claim 4). Sibson teaches that the adaptor molecules preferably comprise oligonucleotides in which single stranded ends of known nucleotide composition are present which is complementary to a predetermined nucleic acid end sequence or end nucleotide so as to permit linkage (pg. 9, lines 15-21)(limitations of Claim 4 and 7). "At least some of the adaptor molecules of the present invention can be structured so as to permit separation of the present process by immobilizing adaptored products on a solid phase (pg. 10, lines 21-25). A population of adaptors may be used simultaneously such that the total possible adaptor molecules required for "adaptoring" all possible sequence types (pg. 14, lines 15-16). Adaptors may carry biotin to allow selective separation of the desired adaptor molecules (pg. 19, lines 1-3). Sibson teaches that it is preferred that adaptors covering all possible reactions in a chosen subset of sequence be present, because then the opportunity for fragments in the chosen subset to ligate to each other is minimized (pg. 20, lines 21-25)(limitations of Claim 8). Sibson also teaches contacting the nucleic acid population with one or more oligonucleotide sequences. Using primers of preselected sequence, effectively enables one or more predetermined subset(s) of sequence to be selected (pg. 11, lines 3-5). Sibson further teaches isolating nucleic acid which correctly

hybridizes to an oligonucleotide sequence by capturing the oligonucleotide sequence on a solid phase (pg. 13, step c). The oligonucleotide sequence has a predetermined recognition sequence. The nucleic acid is categorized by its ability to correctly hybridize to oligonucleotide sequences having the recognition sequence. The recognition sequence is situated such that it recognizes a sequence in the double-stranded portion of the nucleic acid. Finally, one or more different recognition sequences is represented in the oligonucleotide sequence. Sibson discloses a kit comprising adaptors, endonuclease, FoKI, and oligonucleotide sequences used as PCR primers (pg. 23, para. 2 and Claims 29-34)(limitations of Claim 26-28 and 32-35). The PCR primers comprise in some embodiments a sequence complementary to the core sequence of the adaptors ('first sequence") and may preferably extend by one or more specific extra bases into the adaptored fragment ('third sequence")(pg. 28, lines 25)(limitations of Claim 9-11). This implies that the oligonucleotide sequences also contain the sequence of the single stranded portion of the adaptor ("second sequence")(limitations of Claim 5). Thus, these primers contain all the technical features of the oligonucleotide sequences claimed.

Sibson does not explicitly teach using a oligonucleotide which has a pre-determined recognition sequence which is situated in a portion of the nucleic acid which was double-stranded after digestion with the endonuclease.

However, Rothberg teaches that following cDNA preparation, the next step is simultaneous RE cutting of and adapter ligation to the sample cDNA sequences (col. 48, lines 42-44). As seen in Figure 2D the oligonucleotide 222 comprises a segment

complementary to the adaptor, the overhang/sticky end, the restriction endonuclease site and the double stranded nucleic acid (limitations of Claim 5, 8). Rothberg teaches that primers are preferably constructed with a subsequence 226 of P nucleotides. Length P is preferably from 1 to 6 and more preferably either 1 or 2 (col. 51, lines 49-56)(limitations of Claim 9, 10, 11). Rothberg teaches that the primer comprises at the 3' end of and contiguous with the longer strand sequence the portion of the restriction endonuclease recognition site remaining on the nuclei acid fragment terminus after digestion by the restriction endonuclease...contiguous to said one or more additional nucleotides, and optionally such that said primers comprising a particular said one or more additional nucleotides can be distinguishably detected from said primers comprising a different said one or more additional nucleotides (col. 11, lines 20-39). Rothberg teaches why a primer complementary to a portion of the double-stranded nucleic acid is preferable "the joint result of using primers 223 with subsequence 226 in multiple PCR reactions after one RE/ligase reaction is to extend the effective target subsequence from the RE recognition subsequence by concatenating onto the recognition sequence a subsequence which is complementary to subsequence 226 (limitations of Claim 37). Thereby, many additional target subsequences can be recognized while retaining the specificity and exactness characteristic of the RE embodiment (col. 52, lines 6-14).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified method of Sibson to include the oligonucleotide of Rotherberg. The ordinary artisan would have been motivated to have

used an oligonucleotide which has a pre-determined recognition sequence which is situated in a portion of the nucleic acid which was double-stranded after digestion with the endonuclease for the express benefit taught by Rothberg. Rothberg teaches why a primer complementary to a portion of the double-stranded nucleic acid is preferable "the joint result of using primers 223 with subsequence 226 in multiple PCR reactions after one RE/ligase reaction is to extend the effective target subsequence from the RE recognition subsequence by concatenating onto the recognition sequence a subsequence which is complementary to subsequence 226.

7. Claims 14-22, 42-46, 49-60, 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rothberg et al. (US Pat. 5,871,697, February 16, 1999) in view of Dynal Catalog (1995).

Rothberg et al (herein referred to as Rothberg) teaches a method for categorizing nucleic acid by

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein the endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has sticky ends of a known base sequence and of a known common length (col. 9, lines 42-43);

(ii) contacting the nucleic acid population with an adaptor to ligate the adaptor to a termini of each nucleic acid in the population such that the adaptor has a double stranded primer portion having a known base sequence and a single stranded portion

complementary to the known sticky end of the nucleic acids of the population (col. 9, lines 43-56);

(iii) contacting the nucleic acid with one or more oligonucleotide sets (57-60) and

(iv) categorizing the nucleic acid by isolating nucleic acid which correctly hybridizes to an oligonucleotide set, wherein each oligonucleotide sequence in each oligonucleotide set has a pre-determined recognition sequence such that the recognition sequence is situated in the portion of the nucleic acid which was double stranded after digestion with the endonuclease (limitations of Claim 4, 7).

Simply, Rothberg teaches that following cDNA preparation, the next step is simultaneous RE cutting of and adapter ligation to the sample cDNA sequences (col. 48, lines 42-44). As seen in Figure 2D the oligonucleotide 222 comprises a segment complementary to the adaptor, the overhang/sticky end, the restriction endonuclease site and the double stranded nucleic acid (limitations of Claim 5, 8). Rothberg teaches that primers are preferably constructed with a subsequence 226 of P nucleotides. Length P is preferably from 1 to 6 and more preferably either 1 or 2 (col. 51, lines 49-56)(limitations of Claim 9, 10, 11). Rothberg teaches that if necessary, prior to the first step, the cDNA sample is prepared by methods commonly known in the art, such as amplification (col. 47, lines 23-26 and col. 87, lines 15-31)(limitations of Claim 12). Rothberg teaches that the primer comprises at the 3' end of and contiguous with the longer strand sequence the portion of the restriction endonuclease recognition site remaining on the nuclei acid fragment terminus after digestion by the restriction endonuclease...contiguous to said one or more additional nucleotides, and optionally

such that said primers comprising a particular said one or more additional nucleotides can be distinguishably detected from said primers comprising a different said one or more additional nucleotides (col. 11, lines 20-39). Rothberg teaches why a primer complementary to a portion of the double-stranded nucleic acid is preferable "the joint result of using primers 223 with subsequence 226 in multiple PCR reactions after one RE/ligase reaction is to extend the effective target subsequence from the RE recognition subsequence by concatenating onto the recognition sequence a subsequence which is complementary to subsequence 226 (limitations of Claim 37). Thereby, many additional target subsequences can be recognized while retaining the specificity and exactness characteristic of the RE embodiment (col. 52, lines 6-14). Rothberg explicitly teaches that restriction enzymes (RE's) such as those known as class IIS restriction enzymes, which produce overhangs of unknown sequence are less preferable (col. 41, lines 12-15). Rothberg teaches that preferred REs have a 6 pb recognition site and generate a 4 bp 5' overhang. The RE embodiments are also adaptable to a 2 bp 5' overhang, which is less preferred since 2 bp overhangs have a lower ligase substrate activity than 4 bp overhangs (col. 42, lines 5-9). Rothberg specifically teaches that adapter 250 is specific for the RE BamHI, as it has a 3' end complementary to the 5' overhand generated by BamHI (col. 45, lines 64-65). Similarly, Adapter 251 is specific for HindIII.

Rothberg also teaches a kit which contains one or more restriction endonucleasese, adapters and primers of the instant invention (col. 25-26)(limitations of Claim 26-29, 32-36). Rothberg teaches that the primers are detestably labeled such

that primer with differing said one or two additional nucleotides have different labels that can be distinguishably detected (col. 26, lines 30-32).

Rothberg does not specifically teach categorizing the nucleic acid by denaturing the nucleic acid population, immobilizing the nucleic acids, extending the oligonucleotides, denaturing the double stranded nucleic acid, contacting the immobilized single stranded nucleic acid with a second set of oligonucleotides sequences, extending the oligonucleotide, denaturing and isolating the resulting non-immobilized nucleic acids.

However, Dynal teaches a method of generating and isolating non-immobilized single-stranded nucleic acid. Dynal teaches contacting a first set of oligonucleotide sequences, biotinylated primers, with the nucleic acid population. The single stranded primers hybridized, extended via PCR and then immobilized onto a Dynabead via the biotin (Figure 10.1) The double stranded nucleic acid is denatured and the non-biotinylated immobilized species is removed. The immobilized single-stranded nucleic acid is the contacted with a random priming or a specific labeled primer, a second set of oligonucleotide sequence, and extended to form a double-stranded nucleic acid. The double stranded nucleic acid is the denatured and the resulting non-immobilized single stranded nucleic acid is isolated.

Therefore, it would have been **prima facie** obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Rothberg with the teachings of Dynal. The ordinary artisan would have been motivated to have performed the categorizing method of Rothberg and subsequently performed the

method of Dynal to synthesize single-stranded probes in order to generate probes of known sequences in which were identified by the categorization method of Rothberg. Rothberg explicitly teaches that following the RE/ligase step is amplification of the doubly cut cDNA fragments such that any amplification method that selects fragments to be amplified based on end sequences is adaptable (col. 50, lines 5-8). The amplification method of Dynal is based upon the end sequences, thus would be considered an equivalent means of amplifying the cDNA fragments. The ordinary artisan would have been motivated to have amplified and generated nucleic acid from a sample for subsequent analysis in the categorization method. Moreover, it would be obvious to place these added reagents into the kit of Rothberg for the ability to easily perform the assay.

With regard to Claim 18, such that the oligonucleotide is contacted with the solid support prior to the nucleic acid population, it is well known that the primer may be contacted with the solid phase prior to the nucleic acid.

With regard to Claims 19-22, the teachings of Rothberg that oligonucleotides which has predetermined sequences of one or two bases would teach the ordinary artisan to use all of the possible combinations for the expected property of testing all possible combinations.

8. Claims 23-24, 39-40, 47-48, 61-62 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rothberg et al. (US Pat. 5,871,697, February 16, 1999 or Rothberg et al. (US Pat. 5,871,697, February 16, 1999) in view of Dynal Catalog (1995) as

applied to Claims 14-22, 42-46, 49-60, 63 above, and further in view of Hartley et al (US Pat 5,106,727, April 1992).

Rothberg et al (herein referred to as Rothberg) teaches a method for categorizing nucleic acid by

(i) digesting double-stranded nucleic acid with an endonuclease to produce a nucleic acid population, wherein the endonuclease is selected such that each nucleic acid in the resulting nucleic acid population has sticky ends of a known base sequence and of a known common length (col. 9, lines 42-43);

(ii) contacting the nucleic acid population with an adaptor to ligate the adaptor to a termini of each nucleic acid in the population such that the adaptor has a double stranded primer portion having a known base sequence and a single stranded portion complementary to the known sticky end of the nucleic acids of the population (col. 9, lines 43-56);

(iii) contacting the nucleic acid with one or more oligonucleotide sets (57-60) and

(iv) categorizing the nucleic acid by isolating nucleic acid which correctly hybridizes to an oligonucleotide set, wherein each oligonucleotide sequence in each oligonucleotide set has a pre-determined recognition sequence such that the recognition sequence is situated in the portion of the nucleic acid which was double stranded after digestion with the endonuclease (limitations of Claim 4, 7).

Simply, Rothberg teaches that following cDNA preparation, the next step is simultaneous RE cutting of and adapter ligation to the sample cDNA sequences (col. 48, lines 42-44). As seen in Figure 2D the oligonucleotide 222 comprises a segment

complementary to the adaptor, the overhang/sticky end, the restriction endonuclease site and the double stranded nucleic acid (limitations of Claim 5, 8). Rothberg teaches that primers are preferably constructed with a subsequence 226 of P nucleotides. Length P is preferably from 1 to 6 and more preferably either 1 or 2 (col. 51, lines 49-56)(limitations of Claim 9, 10, 11). Rothberg teaches that if necessary, prior to the first step, the cDNA sample is prepared by methods commonly known in the art, such as amplification (col. 47, lines 23-26 and col. 87, lines 15-31)(limitations of Claim 12). Rothberg teaches that the primer comprises at the 3' end of and contiguous with the longer strand sequence the portion of the restriction endonuclease recognition site remaining on the nuclei acid fragment terminus after digestion by the restriction endonuclease...contiguous to said one or more additional nucleotides, and optionally such that said primers comprising a particular said one or more additional nucleotides can be distinguishably detected from said primers comprising a different said one or more additional nucleotides (col. 11, lines 20-39). Rothberg teaches why a primer complementary to a portion of the double-stranded nucleic acid is preferable "the joint result of using primers 223 with subsequence 226 in multiple PCR reactions after one RE/ligase reaction is to extend the effective target subsequence from the RE recognition subsequence by concatenating onto the recognition sequence a subsequence which is complementary to subsequence 226 (limitations of Claim 37). Thereby, many additional target subsequences can be recognized while retaining the specificity and exactness characteristic of the RE embodiment (col. 52, lines 6-14). Rothberg explicitly teaches that restriction enzymes (RE's) such as those known as

class IIS restriction enzymes, which produce overhangs of unknown sequence are less preferable (col. 41, lines 12-15). Rothberg teaches that preferred REs have a 6 bp recognition site and generate a 4 bp 5' overhang. The RE embodiments are also adaptable to a 2 bp 5' overhang, which is less preferred since 2 bp overhangs have a lower ligase substrate activity than 4 bp overhangs (col. 42, lines 5-9). Rothberg specifically teaches that adapter 250 is specific for the RE BamHI, as it has a 3' end complementary to the 5' overhand generated by BamHI (col. 45, lines 64-65). Similarly, Adapter 251 is specific for HindIII.

Rothberg also teaches a kit which contains one or more restriction endonucleases, adapters and primers of the instant invention (col. 25-26)(limitations of Claim 26-29, 32-36). Rothberg teaches that the primers are detestably labeled such that primer with differing said one or two additional nucleotides have different labels that can be distinguishably detected (col. 26, lines 30-32).

Rothberg does not specifically teach the incorporation of analogues into the oligonucleotides.

However, Hartley et al. (herein referred to as Hartley) teaches incorporating non-standard bases into random primers to reduce de novo synthesis.

Therefore, it would have been **prima facie** obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Rothberg to include the non-standard bases as taught by Hartley for reducing the de novo synthesis. The ordinary artisan would have been motivated to have reduced the amount of de

novo synthesis to obtain results representative of the categorized population as opposed to additional nucleic acid molecules.

Conclusion

9. No claims allowable over the art.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Enewold Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Thursday from 7:00AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Jeanine Enewold Goldberg
May 17, 2001

JEFFREY FREDMAN
PRIMARY EXAMINER